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(57) Abstract

The present invention describes an enzymatical method for the treatment of soy suspensions, to obtain a suspension with a low waterbinding capacity and a low viscosity. The treatment is accomplished using a limited amount of critical enzymes. A soy composition containing a reduced amount of monosaccharides and uronic acids is obtained.

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Enzymatic treatment of soy

Technical field

The present invention relates to a method for deviscosifying soy suspensions, whereby deviscosification is accomplished using a limited number of pure enzymes. The reactions are performed in such a way that the soy becomes less viscous without affecting the nutritional value of the product. The waterbinding capacity of the soy is also reduced.

Background of the invention

Soy beans are a cheap source of protein. These proteins can be used among others as milk replacers in food and feed applications.

It has been recognized in European patent application EP 479 596 that soybean meal or flour forms a highly rigid slurry in an aqueous suspension. This highly viscous slurry is difficult to process and it is therefore necessary to utilize highly dilute slurries, generally below 15 % by weight. Alternatively, the water unsoluble solids fraction, representing the main part of the waterbinding and viscosity causing factors can be removed by centrifugation.

Spraydrying of dilute slurries is non economical and preconcentration by film evaporation is indispensable. However, it is not possible to film evaporate the soy slurry to desirable concentrations of about 40-50% dry solids, due to the high viscosity of the slurry. Apart from being disadvantageous during industrial processing, high viscosities have also been identified as a major nutritional

constraint in the feeding of broilers and pigs (e.g. Feedstuffs, February 1, 1993, 14-15).

Several patent documents, including DE 1 792 142, US 4,119,733 and EP 479 596, disclose the use of crude enzyme mixtures to reduce the viscosity of the slurry before further drying. These complex enzyme mixtures contain among others cellulase, hemicellulase, pentosanase, ß-glucanase, xylanase, cellobiase, pectinase, invertase, arabinanase, glucoamylase, α-galactosidase and proteases. Such undefined enzyme mixtures are thought to be necessary to hydrolyse the many polymeric compounds known to be present in soy beans. Especially the so-called Non-Starch-Polymer (NSP) fraction of soy is known to be complex (see for example Carré & Brillouet, J. Sc. Food Agricult. 37 (1986), 341-351) and is implicated in the waterbinding properties and viscous character of aqueous soy suspensions.

Unfortunately, the use of crude, non-defined enzyme mixtures to degrade the complex NSP fraction of soy will lead to the generation of undesirable degradation products. Some of these degradation products are known to be disadvantageous in feed applications (Longstaff et al, British Poultry Science 29 (1988), 379-393; Schutte et al, British J. Nutr. 68 (1992), 195-207; Schutte et al, British Poultry Science 33 (1992), 89-100), like for example uronic acids (generated by pectinolytic enzyme activities), xylose (generated by xylanolytic enzyme activities), or arabinose (generated by arabinanolytic enzyme activities).

Because of the generation of these unwanted side-products, the use of complex enzyme mixtures to reduce waterbinding and viscosity of soy suspensions is less desirable. This is also true for applications where the enzyme is added to feed and intended to act in the gastro-intestinal tract of an animal (like calfs, pigs and poultry).

Although for some crops, like wheat or barley, the viscosity causing components have been identified, the situation in soy is much more complex. Soy is known to contain the following components: proteins, hemicelluloses,

celluloses, pectins and oil. The effects of each of these individual compounds on viscosity and waterbinding properties of the soy slurry is largely unknown.

The viscosity reducing agents suggested in DE 1 792 142, US 4,119,733 and EP 479 596 are enzymes belonging to the group of carbohydrase enzymes. The carbohydrase enzymes contain several activities e.g. cellulase, hemicellulase, pectinase, xylanase, invertase, endo-glucanase, cellobiase, arabinanase and the like. In the indicated patent documents, crude mixtures of the indicated activities are used. This implicates nonoptimal amounts of the indicated enzyme activities. By following this approach, high enzym costs, suboptimal lowering of viscosities and waterbinding capacities and, moreover, undesirable side-products can be expected.

Therefore, the availability of a limited set of pure enzymes by which the soy suspension could be selectively and cost-effectively modified in terms of waterbinding, viscosity and side-products would be desirable.

Summary of the invention

The present invention discloses the use of a limited number of enzymes for the treatment of soy bean slurries.

An enzyme mixture is disclosed for the treatment of an aqueous suspension of soy meal, comprising enzyme activities selected from the group comprising cellulolytic, hemicellulolytic and pectinolytic activities, whereby the enzyme mixture comprises a limited number of critical enzymes.

Preferably, the mixture comprises one or more of the following critical enzymes: endo-glucanases, cellobiohydrolases, endo-arabinanases, arabinofuranosidases, endo-pectinases, endo-xylanases and endo-galactanases. More preferably, the mixture comprises one or more of the following critical enzymes: endo-glucanases, endo-

arabinanases and endo-galactanases.

The present invention discloses a specific mixture comprising the following enzymes: endo-glucanase V, cellobiohydrolase III, endo-arabinanase, arabinofuranosidase B, pectin lyase, endo-xylanase and endo-galactanase. Another specific mixture comprises the enzymes endo-glucanase V, endo-arabinanase and endo-galactanase.

The present invention further discloses enzyme mixtures wherein the critical enzymes are mixed in an optimized ratio.

Optionally the enzyme mixture of the present invention also contains a protease.

The mixtures of the present invention are especially useful to obtain a reduction in viscosity and/or a decrease in the waterbinding capacity of an aqueous soy suspension.

The present invention further discloses a process for the preparation of a soy composition comprising a treatment of a soy slurry with the enzyme mixture of the present invention.

The enzyme mixtures of the present invention enable the preparation of a soy composition with levels of uronic acid and monosaccharides which are comparable to the levels occurring in non-enzymatically obtained soy compositions.

Detailed description of the invention

The present invention discloses the use of a limited number of polymer-degrading enzymes for the treatment of soy bean slurries. Specifically, the invention discloses enzymatic mixtures for treatment of an aqueous suspension of flaked or milled soy. The soy meal is optionally defatted prior to the addition of water.

Soy bean slurries are enzymatically treated to obtain a reduction in viscosity and/or a decrease in waterbinding capacity of the soy slurry. A reduction in viscosity is essential to achieve cost effective drying of the final product.

The present invention discloses specific enzyme mixtures for treatment of an aqueous suspension of defatted or non-defatted soy meal. It is demonstrated by the present invention that the viscosity and the waterbinding capacity of a soy preparation can be reduced using a limited number of enzymes.

The enzyme mixture used in the present invention comprises enzymes which are selected from the group comprising cellulolytic, hemicellulolytic and pectinolytic enzymes. The enzyme mixture contains only a limited number of enzymes, the so-called critical enzymes. Critical enzymes in this respect are enzymes which selectively destroy the structure of particular soy components, i.e. polymers. Hence bound water is released and/or a reduction in the viscosity of the soy product is achieved, without the concomitant production of unwanted side products.

Critical enzymes are those enzymes which are able to attack the cellulose, hemicellulose and/or pectin polymers present in soy, without the generation of substantial amounts of monomeric degradation products. Specifically, critical enzymes are endo-acting enzymes, i.e. enzymes which release oligomeric products from a polymeric backbone.

The present invention discloses a mixture of enzymes whereby the enzymes are selected from the group comprising: endo-pectinases, endo-galactanases, endo-arabinanases, arabinofuranosidases, endo-xylanases, endo-glucanases and cellobiohydrolases. Enzymes contained within this mixture are suitable to effectively destroy the structure of particular soy polymers. The present invention further discloses a mixture of enzymes whereby the enzymes are selected from the group comprising: endoglucanases, endoarabinanases and endo-galactanases.

A specific enzyme mixture contains the following enzymes: endo-glucanase V, cellobiohydrolase III, endo-arabinanase, arabinofuranosidase B, pectin lyase, endo-xylanase and endo-galactanase. A particularly suitable enzyme mixture contains the following enzymes: endo-glucanase V,

endo-arabinanase and endo-galactanase.

Optionally, the mixtures of the present invention also contain a protease.

It is a further aspect of the invention to use the critical enzymes in optimized ratios, whereby the activities of the individual enzymes are determined and the enzymes are mixed in optimal amounts.

Each individual critical enzyme constitutes at least 20%, preferably at least 30%, more preferably at least 40% of the amount of protein present in the preparation containing said individual enzyme.

The enzymes of the present invention are obtainable from several different microorganisms, preferably they are obtainable from fungi such as <u>Aspergillus</u>, <u>Trichoderma</u> and <u>Penicillium</u>. Preferred species include <u>Aspergillus niger</u>, <u>Aspergillus tubigensis</u>, <u>Aspergillus oryzae</u>, <u>Aspergillus aculeatus</u>, <u>Trichoderma longibrachiatum</u>, <u>Trichoderma reesei</u>, <u>Penicillium emersonii</u>, <u>Penicillium funicullosum</u> and <u>Humicola insolens</u>.

The microorganism are cultured under conditions conducive to production of the desired enzymes. In particular, a specific inducing substance can be used in the fermentation broth, which will lead to the preferred synthesis of the desired enzyme in large quantities. The enzymes suitable to treat soy preparations according to the present invention are purified from a crude microbial fermentation broth using protein purification techniques known to the skilled person.

In another embodiment of the invention, suitable enzymes are obtained by subjecting a commercial enzyme preparation to protein purification techniques.

Specifically, a crude fermentation broth or a commercial enzyme preparation is fractionated, whereby the performance of individual fractions or combinations thereof on a soy suspension can be monitored. In this way, fractions displaying the proper performance can be selected.

In a preferred embodiment of the invention, a gene

encoding an enzyme of interest is isolated, cloned and (over)expressed in a desired microbial host organism. Preferably, the desired host organism does not at the same time produce large quantities of other enzymes. Suitable host organisms can be found among yeasts and fungi. Preferred hosts are found among strains belonging to the yeast genera Saccharomyces and Kluyveromyces and the fungal genus Aspergillus. (Over)expression of a gene encoding a desired enzyme is especially advantageous because in that way high expression levels of the enzyme of interest are guaranteed, sometimes exceeding 10 g enzyme/l fermentation broth.

The enzyme mixture of the present invention optionally contains a protease. Proteases are obtainable from, for example, Aspergilli and Bacilli. Preferred species include Aspergillus oryzae, Aspergillus niger, Aspergillus tubigensis, Bacillus subtilis and Bacillus licheniformis.

The process in which the enzymatic mixture is used comprises the following steps:

a) the dehulled soy beans are subjected to a wet milling operation, optionally water is directly added to soy flour to reach the desired dry matter content,
b) optionally the wet slurry is heat-treated to destroy anti-nutritional factors or enzymatic activities, and subsequently cooled,
c) optionally the pH of the slurry is adjusted,
d) the slurry is enzymatically hydrolysed.
e) optionally, the heat-treatment, cooling and enzyme treatment is repeated.

The enzyme mixture is added to the slurry in an amount of 0.001-1%, preferably 0.01-0.1% (w/w) enzyme.

The reaction is performed between 45 and 75°C, more preferably the temperature is between 50 and 60°C. The pH of the mixture is between 3.5 and 8.5, preferably the reaction is performed between a pH of 4.5 and 7.0. Obviously, the choice of the pH will influence the activities of the enzymes present in the mixture.

The reaction is continued until the waterbinding

capacity and the viscosity of the soy preparation are lowered so that it becomes possible to handle a slurry containing an amount of 30-50% (w/w) of solids after film evaporation. Adequate deviscosification can be checked using known devices such as an Haake viscosimeter or a Rotafab. A centrifugation test was found to be indicative for a reduction in waterbinding capacity.

The soy composition obtained after application of the process of the present invention can be used as a food or feed supplement. The soy composition exhibits an improved water solubility and has an improved digestibility. The formation of uronic acids or monosaccharides like xylose and arabinose is minimized.

The soy composition obtained after the process of the present invention can, for example, be employed in a milk replacer intended for human or animal use.

The present invention is demonstrated by several examples. In Example 1 a method for preparing a soy suspension is disclosed. A soy suspension obtained according to this method is used in the subsequent examples.

Example 2 and 3 describe the effect of, on the one hand, a complex commercial enzyme preparation and, on the other hand, two well-defined mixtures of several (partially) purified enzymes, on the waterbinding capacity of the soy suspension. It should be noted that the activities of the enzymes are strongly pH dependent. Alteration of the conditions, e.g. the pH of the incubation mixture or the use of a defatted instead of a non-defatted soy suspension, may lead to a different selection of enzymes to include in the preferred mixture.

In Example 4 a comparison is made of the performance of a three-enzyme mixture containing the enzymes in an optimized ratio with the performance of a commercial enzyme preparation, specifically developed for soy processing. The three-enzyme mixture is performing quite well on a complex substrate like soy.

Example 5 shows that the addition of a relatively low

amount of protease has an positive effect on reduction of viscosity, without further affecting the waterbinding capacity of a soy suspension.

Example 1
Process for the preparation of a soy suspension

The soy suspension was essentially produced as described in US 4,194,018.

To dehulled and presoaked soy beans, water is added with a temperature of 90°C in a ratio of 4 parts water per 1 part soy beans. This mixture is sequentially introduced into a series of Fryma mills and milled to obtain a particle size of 90% of the particles of less than 500 μ. This milling procedure is conducted rapidly so as to avoid excessive mechanical heat generation. If necessary, the milling step can be repeated. Subsequently, the milled slurry is introduced into a continuous Dyno ball mill to obtain the required particle size.

To mimic the trypsin inhibitor inactivation process, the milled slurry is immediately introduced into a jet cooker where the slurry is subjected to a heat treatment of 2-4 minutes at 120-140°C. After passing the counter pressure valve, the slurry is collected in a sterile container and stored at 4°C. Batches used for the subsequent enzymatic hydrolysis experiments typically have a dry substance content between 12-16%; if required batches were subjected to a rotavap evaporation step to obtain the required dry substance concentration.

Example 2
Waterbinding capacity of soy suspension treated with a complex mixture of carbohydrases

About 10 gram of an aqueous soy suspension (Example 1) with a dry solid content of 12.2 % is mixed with Rapidase® Press to homogeneity. Rapidase® Press is a commercial enzyme

preparation from Gist-brocades (the Netherlands). The preparation is obtained from Aspergillus niger and represents a complex mixture of cellulolytic, hemicellulolytic and pectinolytic enzymes. The proteolytic activity of the enzyme preparation is low. Before adding the enzyme, the pH of the soy suspension is adjusted to the desired value by adding 4 N hydrochloric acid. The enzymatic reaction proceeds for three hours during which the temperature of the soy suspension in the closed vessel is kept at 55°C by means of indirect heating. The homogeneous mixture of soy suspension and enzymes is not stirred during the enzymatic treatment at 55°C. The waterbinding of the incubated soy suspension is established by measurement of the weight the supernatant, obtained after centrifugation of the incubated soy suspension during 10 minutes at 28.000 g and 35°C.

Table 1. Liquid release of a soy suspension using a complex Aspergillus niger enzyme mixture (Rapidase® Press).

Total protein/Soy suspension	Liquid release
0	-
0.002	0.059
0.024	0.085
0.060	0.103
0.179	0.126
0.357	0.139

Rapidase® Press is added to soy suspension adjusted to pH 6.0. The dosage varies between 0.002 and 0.357 protein weight percent per weight of soy suspension as such. Table 1 shows the liquid release after incubation with various dosages of Rapidase® Press. The liquid release is calculated from the weight of supernatant per weight of incubated soy suspension, minus the weight of a non-enzymatically treated control supernatant. This control supernatant is obtained by incubation of soy suspension with the same volume of demineralised water as the volume of the added enzyme soluti-

on, and subsequent centrifugation. The weight of control supernatant per weight of non-enzymatically treated soy suspension is 0.470 g/g.

Table 1 shows that the enzyme treatment leads to a reduction of the waterbinding capacity of the soy suspension.

Example 3
Effect of purified carbohydrases on the waterbinding capacity of soy suspension

To test the performance of more defined enzyme mixtures, several enzymes were either biochemically purified or overexpressed by molecular cloning procedures, and subsequently tested in the reduction of waterbinding capacity.

The (partially) purified enzymes used in the experiment were the following:

* Endo-glucanase V (EC 3.2.1.4 as described by Beldman et al. (Eur. J. Biochem. 146 (1985), 301-308), this is not the standard endo-glucanase) was purified from Maxazyme® CL 2000 (Gist-brocades, the Netherlands) according to Beldman et al. (op. cit.). After purification, the enzyme was concentrated by ultrafiltration on a Filtron membrane (cut off 10 kD) to a protein concentration of 48.2 mg/ml.
* Cellobiohydrolase III (EC 3.2.1.91) from Trichocherma viride was also purified from Maxazyme® CL 2000 according to the method of Beldman et al. (op. cit.). After purification, the enzyme fraction containing CBHIII was concentrated by ultrafiltration on a Filtron membrane (cut off 10 kD) to a protein concentration of 108.6 mg/ml.
* Endo-arabinanase (EC 3.2.1.99) was obtained from A. nidulans strain G191 transformed with the abnA gene from A. niger (EP-A-0506190). Material from strain G191::pIM950-170, designated ABN102, was used for this study. Strain ABN102 was grown for 40 hrs at 30°C in 2 l

shake flasks containing 0.5 l medium. The medium contains per liter: 10 g sugarbeet pulp, 1 g yeast suspension, 15 g potassium dihydrogen phosphate, 4 g ammonium chloride, 0.5 g magnesium sulphate, 0.5 g potassium chloride, 1 ml Vishniac solution. Vishniac solution contains per 100 ml: 0.44 g zinc sulphate hepta-hydrate, 0.1 g manganese chloride tetra-hydrate, 0.03 g cobalt chloride hexa-hydrate, 0.03 copper sulphate penta-hydrate, 0.025 g disodium molybdate dihydrate, 0.14 g calcium chloride dihydrate, 0.1 g ferrous sulphate hepta-hydrate, 1.0 g EDTA. The pH of the medium was adjusted to 6.0 with 1 N KOH.

After fermentation the medium was made germ-free by filtering successively over the following filters: 1. filter paper (Buchner-funnel); 2. glass-fibre filter (Whatmann GF/A or GF/B); 3. hardened filter circles (Whatmann); 4. 0.45 μm membrane filter (Schleicher & Schuell; 5. 0.2 μm membrane filter (Schleicher & Schuell). The sterile fermentation supernatant was further concentrated by ultrafiltration on a Filtron membrane (cut off 10 kD) to a protein concentration of 12.2 mg/ml.

* Arabinofuranosidase B (EC 3.2.1.55) was produced by Aspergillus niger strain N593 transformed with multiple copies of the abfB gene from A.niger (EP-A-0506190) under control of the amyloglucosidase promoter from A. niger. The best-producing transformant, designated N593-T8 was grown as described in EP-A-0506190.

  The fermentation supernatant was made germ-free as described for endo-arabinanase and concentrated by ultrafiltration on an Amicon filter type YM10 (cut off 10kD) and freeze-dried.

  Before use, arabinofuranosidase B was dissolved in water to a protein concentration of 118.9 mg/ml.

* Pectin lyase (EC 4.2.2.10) was purified from Rapidase® Press by the following method:

  The pH of the fermentation broth was adjusted to 4.0

with acid and bentonite was added to a concentration of 3% (v/v), followed by recovery of the liquid phase by centrifugation or filtration. After the liquid phase was adjusted to pH 6.0, pectin lyase could be purified by chromatography on Whatmann QA-cellulose/DS 29. After loading of the enzyme, the column was washed with 0.02 M phosphate buffer pH 6.0, containing 0.2 M NaCl. Pectin lyase was eluted with the same buffer containing 0.3 M NaCl. After purification the enzyme was concentrated as described under arabinofuranosidase B to a concentration of 14.5 mg/ml.

* Endo-xylanase I (EC 3.2.1.8) was isolated from <u>A. niger</u> CBS 513.88 transformed with plasmid PXYL3AG containing the xylanase gene under control of the <u>A. niger</u> amyloglucosidase promoter as described in EP-A-0463706. The strain was grown as described in EP-A-0463706 and the fermentation supernatant was made germfree as described for endo-arabinanase.
  The supernatant was concentrated by ultrafiltration on a Filtron membrane (cut off 10 kD) and freeze-dried. Before use, endo-xylanase was dissolved in water to a concentration of 72.0 mg/ml.
* Endo-galactanase (EC 3.2.1.89) was obtained from Megazyme Ltd. (Australia). The preparation has a specific activity of 408 U/mg and a protein concentration of 1.08 mg/ml. Prior to use, the enzyme was extensively dialyzed to remove excess salt.

Using the various purified enzyme preparations, two mixtures were prepared containing:

<u>Mixture I</u>

- Endo-glucanase V
- Cellobiohydrolase III
- Endo-arabinanase
- Arabinofuranosidase B
- Pectin lyase
- Endo-xylanase I
- Endo-galactanase

Mixture II

- Endo-glucanase V
- Endo-arabinanase
- Endo-galactanase

Enzyme mixtures I and II were added to soy suspension adjusted to pH 6.0. The dosage was 0.001 protein weight percent for each enzyme in the mixture per weight of the soy suspension as such. Soy suspension incubation and centrifugation conditions were identical to those mentioned in Example 2. The data provided in Table 2 show the liquid release after incubation with the specified enzyme mixtures. The liquid release was calculated as indicated in Example 2. The weight of control supernatant per weight of non-enzymatically treated soy suspension is 0.535 g/g.

Table 2. Liquid release of a soy suspension using mixtures of selected purified enzymes.

Enzyme mixture	Total protein/Soy suspension	Liquid release
I	0.007	0.079
II	0.003	0.089

Table 2 clearly demonstrates that the liquid release obtained after incubation of soy suspension with Enzyme Mixtures I and II is larger than the liquid release obtained with the complex enzyme mixture Rapidase® Press, when equal total protein dosages are compared. Especially the fact that these results can be obtained by the proper combination of just three enzymes (Mixture II) illustrates the competetiveness of the approach.

Example 4

Effect of an optimized mixture of purified carbohydrases on the waterbinding capacity of soy suspension

Data provided in Example 3 indicate that a mixture of only three purified enzymes can effectively compete with a

complex mixture of carbohydrase enzymes in destroying the waterbinding capacity of soy suspension.

This example describes a further optimization of the enzymes using a mixture design experiment (J.A. Cornell in: Experiments with Mixtures; eds. John Wiley Interscience) keeping the total protein content of the enzymes added constant.

Starting from the endo-glucanase V, endo-arabinanase and endo-galactanase enzym preparations described in Example 3, different mixtures were prepared.

Each one of these mixtures was added to soy suspension (d.m. 12.2%), pH 6.0 and incubated at 55°C for 3 hours. After incubation, the mixtures were centrifuged for 10 minutes at 35°C and 28.000 g whereupon the weight of the supernatants was determined. After analysis it turned out that a mixture of 82 ppm endo-glucanase V, 1082 ppm endo-arabinanase and 393 ppm endo-galactanase represented the mix yielding the highest level of supernatant.

In a subsequent experiment this optimized mix was prepared and its effect on the waterbinding capacity of soy suspension was compared with the addition of 1 vol.% Viscozyme (commercially available from NOVO, Denmark). The latter enzyme preparation is positioned as one of the most efficient products for decomposing soya soluble polysaccharides.

Table 3. Liquid release of a soy suspension using an optimized three-enzyme mixture or Viscozyme.

Enzyme added	Liquid release
none	-
3-enzyme mixture (1570 ppm protein)	0.172
Viscozyme 1 vol% (900 ppm protein)	0.207

The data obtained after incubation of soy suspension with the optimized mix or with Viscozyme, and subsequent

centrifugation are indicated in Table 3. The liquid release was calculated as indicated in Example 2. It can be seen that the three-enzyme mixture is surprisingly active in degrading the waterbinding capacity of this very complex soy substrate.

Example 5

Effect of carbohydrases and proteases on waterbinding and viscosity of soy suspension

The enzyme mixtures used are the carbohydrase mixture Rapidase® Press (see Example 2) and the protease preparation Maxazyme® FNS. This acid protease product is derived from an *Aspergillus oryzae* fermentation and is also commercially available from Gist-brocades.

About 10 g of a soy suspension with a dry solid content of 12.2% was prepared according to the procedure described in Example 1.

Before adding the enzyme mixtures, the pH of the soy suspension was adjusted to 5 by adding 4 N hydrochloric acid. The dosage of Rapidase® Press varies between 0.001 and 0.053 protein weight percent per weight of soy suspension as such. In some experiments the Rapidase® Press dosages are supplemented with Maxazyme® FNS. In all cases the Maxazyme® FNS dosage amounts 0.006 protein weight percent per weight of soy suspension as such. Soy suspension incubation and centrifugation conditions were identical to those mentioned in Example 2.

Waterbinding of the incubated soy suspension is measured as indicated in Example 2. Viscosity of the soy suspension is measured at 55°C according to the viscomate procedure. This procedure is based on the measurement of the pressure drop across a capillary (diameter of 0.68 mm; length of 35 mm), upon a constant and reproducible volume displacement by the Gilson diluter type 401. The Gilson automatic sample processor 222 is used for complete automation of the procedure. The viscosity data (peakheight) obtained represent relative vlaues only. To obtain absolute viscosity data, the

equation of Poiseuille can be applied.

As is clear from Table 5 the protease is able to reduce the viscosity in the presence of a sufficient amount of carbohydrases, while waterbinding (as measured by the centrifugation assay, see Example 2) is not reduced by the protease in the presence of carbohydrases.

Table 5. Liquid release and viscosity reduction of a soy suspension using carbohydrase all or not supplemented with protease.

Enzyme mixture	Total protein/Soy suspension	Liquid release	Viscosity (peakheight)
No enzyme	-	-	>14
Rapidase P	0.001	0.125	>14
Rapidase P	0.018	0.146	>14
Rapidase P	0.036	0.174	>14
Rapidase P	0.053	0.214	>14
Rapidase P + Max. FNS	0.001 0.006	0.093	>14
Rapidase P + Max. FNS	0.018 0.006	0.099	>14
Rapidase P + Max. FNS	0.036 0.006	0.152	9.8
Rapidase P + Max. FNS	0.053 0.006	0.206	8.1

These data illustrate that high efficiencies in film evaporation equipment can be obtained only if enzymes required to minimize the waterbinding capacity of the soy suspension are combined with relatively low doses of a suitable protease to minimize the viscosity of the suspension.

Claims

1. An enzyme mixture for the treatment of an aqueous suspension of soy meal comprising enzymes selected from the group comprising cellulolytic, hemicellulolytic and pectinolytic enzymes, characterized in that the enzyme mixture comprises a limited number of critical enzymes.

2. An enzyme mixture according to claim 1, characterized that the critical enzymes are selected from the group comprising: endo-glucanases, cellobiohydrolases, endo-arabinanases, arabinofuranosidases, endo-pectinases, endo-xylanases and endo-galactanases.

3. An enzyme mixture according to claim 2, characterized in that the critical enzymes are selected from the group comprising: endo-glucanases, endo-arabinanases and endo-galactanases.

4. An enzyme mixture according to claim 1, characterized that the enzyme mixture comprises the following critical enzymes: endo-glucanase V, cellobiohydrolase III, endo-arabinanase, arabinofuranosidase B, pectin lyase, endo-xylanase and endo-galactanase.

5. An enzyme mixture according to claim 4, characterized in that the enzyme mixture comprises the following critical enzymes: endo-glucanase V, endo-arabinanase and endo-galactanase.

6. An enzyme mixture according to any one of the preceeding claims, characterized that the critical enzymes are mixed in an optimized ratio.

6. An enzyme mixture according to any one of the preceeding claims, characterized that the enzyme mixture further contains a protease.

7. A process for the preparation of a soy composition by enzymatic treatment of an aqueous suspension of soy meal, characterized in that an enzyme mixture according to any one of the preceeding claims is used.

8. A soy composition obtained by a process including an enzymatical treatment, characterized in that it contains a level of monosaccharides or uronic acids which is comparable to the level occurring in a soy composition obtained by a non-enzymatical process.

INTERNATIONAL SEARCH REPORT

Interr   ıal Application No
PCT/EP 95/01685

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A23L1/211

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A23L A23C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP-A-0 479 596 (CARGILL B.V.) 8 April 1992 cited in the application see page 3, line 38 - page 4, line 16 see page 5, line 5 - line 24; examples 1-7 see claims 1-3,6-8,13-15	1-7
X	DE-A-22 61 177 (ETABLISSEMENTS URY & CIE. ET AL.) 20 June 1973 see example 1	1-3,6,7
X	DE-A-17 92 142 (RÖHM G.M.B.H.) 21 October 1971 cited in the application see claims 1-3; example 1	1-3

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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Date of the actual completion of the international search	Date of mailing of the international search report
16 August 1995	05.09.95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Alvarez Alvarez, C

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INTERNATIONAL SEARCH REPORT

Intern al Application No
PCT/EP 95/01685

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US-A-4 119 733 (DEAN SHUI-TIEN HSIEH) 10 October 1978 cited in the application see claims 1,2,4; example 2 ---	1-3
X	US-A-3 941 890 (FREDERICK G. DRACHENBERG) 2 March 1976 see column 4, line 21 - line 24 ---	1
A	PATENT ABSTRACTS OF JAPAN vol. 134 no. 14 (C-635) ,13 September 1989 & JP,A,01 153056 (TOSHIICHI HARADA) 15 June 1989, see abstract ---	
A	PATENT ABSTRACTS OF JAPAN vol. 124 no. 46 (C-546) ,24 November 1988 & JP,A,63 169973 (MITSUBISHI KASEI CORP.) 13 July 1988, see abstract -----	

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Interr ...al Application No
PCT/EP 95/01685

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